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*STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC SUBSTANCES. IV. VARIATIONS AMONG ACTINOMYCETES, WITH SPECIAL REFERENCE TO ACTINOMYCES GRISEUS**

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Introductory and Historical.—Every student of the actinomycetes has been impressed by the striking variations which these organisms undergo in culture. Some of these variations have long been recognized to be quantitative in nature, whereas others are also qualitative. These variations are found to occur in the pigment production by the culture, in the intensity of the pigment, as well as in its specific nature; also whether it is dissolved in the medium or retained in the mycelium. Further variations are found in the production of enzyme systems, especially proteases and diastases, and in other cultural and physiological characteristics. The formation of aerial mycelium and the manner of sporulation of the culture are among the most significant variable factors. These variation phenomena are further complicated by the fact that frequently sectors or saltants are produced which differ from the rest of the colony with respect to the formation of aerial mycelium or pigmentation; upon isolation, such sectors have given cultures that have often been considered as variants.

These variations have caused considerable confusion in the recognition of fixed species or types for the characterization and classification of this group of organisms. They have even led certain investigators[5, 8] to question the constancy of many actinomyces types as recognized by species descriptions. In illustrating this phenomenon, Waksman[8] directed attention, as far back as 1919, to the variability in the proteolytic mechanism of *Actinomyces griseus*, the organism that forms the subject of this paper. One of the more recent students of variations among actinomycetes[4] came to the conclusion that the morphological and physiological properties of these organisms, when cultured under the same environmental conditions, are constant. The changes observed were believed to be either transitory

modifications, or quantitative rather than qualitative variations, long-time changes being produced under the influence of altered environmental conditions. In addition to these, however, sudden changes of a permanent and hereditary nature were obtained without apparently being affected by the environment. These new forms or variants, which were believed to be rather rare among actinomycetes, differed from the original cultures in various morphological, cultural and physiological properties.

Among the variations reported for actinomycetes, the lytic activities of certain strains deserve attention. Dmitrieff and Souteeff[1] studied a culture apparently belonging to the *Streptomyces* group and designated as *Actinomyces bovis* Bostroem. This culture underwent lysis in liquid broth and on solid media. On agar media, lysis was associated only with a certain type of colony; this phenomenon was always accompanied by the formation of daughter colonies. Among these, two types were recognized: one did not differ from the mother colony and preserved the capacity for lysis; the second did not lyse and differed from the first in morphology. The lysing forms possessed stronger proteolytic properties and apparently did not produce any aerial mycelium; the non-lysing colonies were less proteolytic, formed a chalky aerial mycelium, and changed the reaction of litmus milk to alkaline. Lysis took place in broth cultures in 2 to 3 weeks. It was associated with the living culture and was of the nature of a non-enzymatic and non-transmissible lytic factor.

Other investigators[2] as well found that the lytic factor of actinomycetes is not comparable to bacteriophage or to lysozyme, since it dissolved both dead and living cells, and was strictly species specific; it was also thermostable. The fact that lysis may be limited to certain actinomycetes only when grown upon special media was brought out by Katznelson[3] for a thermophilic organism. This organism changed the reaction of the medium to acid, the addition of $CaCO_3$ to the medium inhibiting lysis. This lytic agent was also non-transmissible.

Experimental.—In these experiments, a strain of *A. griseus* found[4] to be capable of producing the antibiotic substance streptomycin was used. In the hope that strains having greater antibiotic potency than the original culture might be obtained, an effort was made to isolate distinct colonies. Spore suspensions of the organism were plated out on different media, with the dilutions sufficiently high to allow the development of only a few isolated colonies per plate. Incubation took place at 28°C. for 3 to 6 weeks.

The individual colonies developing on the plates showed considerable variation. Some were wrinkled, others were smooth; some produced pure white aerial mycelium, but the majority were characterized by the typical grayish-green color of the mycelium. A few of the colonies were almost completely free of aerial mycelium, and were somewhat moist and glisten-

ing in surface appearance. In some cases sectors were produced which differed from the rest of the colony. Upon transfer to fresh agar media, certain sectors gave rise to growth having a rough or a smooth surface of the typical sporulating aerial mycelium; other sectors, however, were entirely free of aerial mycelium, and produced cultures stable in this respect. The color variation of the aerial mycelium did not appear to be very significant. Some of the sectors from white colonies gave, on further cultivation, the typical gray-green color characteristic of the aerial mycelium of *A. griseus;* in other cases, the color change was reversed.

The various types of colonies and some of the different sectors were transferred to agar media. A number of cultures were thus obtained. These were inoculated into flasks containing nutrient glucose broth, and incubated at 28°C. Some flasks were shaken continuously in order to produce submerged growth, whereas others were kept stationary. The antibiotic activity of the filtrates was measured after different periods of incubation.

The results presented in table 1, as well as other data not reported here,

TABLE 1

PRODUCTION OF STREPTOMYCIN BY DIFFERENT COLONIES AND SECTOR ISOLATES OF *A. griseus*

CULTURE NO.	ORIGIN OF CULTURE	UNITS/ML. OF FILTRATE, AFTER DAYS — SHAKEN CULTURES				STATIONARY CULTURES		
		3	5	6	8	8	12	14
1	Typical sporulating colony	30	44	55	25	98	214	203
2	White sector of above colony	34	36	45	36	64	162	128
4	Non-sporulating sector of a typical colony	0	0	0	0	0	0	0
7	Large pure gray colony	31	30	83	33	45	136	135

indicate that in general the sporulating colonies, whether producing white, gray or gray-green mycelium, were active producers of streptomycin. The non-sporulating strains obtained from asporogenous sectors, however, were inactive. These non-sporulating strains produced no appreciable surface growth in stationary liquid cultures. The glucose consumption was much less, as compared with the active strains. The non-sporulating strains gave a lower pH value of the medium, namely, 5.0 to 6.5, whereas the filtrates of the sporulating types were always alkaline, ranging from pH 7.5 to 8.5. These and other physiological differences pointed to the fact that the non-sporulating forms were markedly different from the sporulating cultures. The two could have been considered as distinct species, had they been isolated separately from a natural substrate.

There was still a possibility that the inactive strains may not have lost the capacity of producing streptomycin, but that this was neutralized by the simultaneous elaboration of an inhibiting substance. The following

experiments tried to eliminate such possibility. Two samples of purified streptomycin of known activity were dissolved, one in water and the other in an equal amount of a glucose-free culture filtrate of an inactive strain. These two solutions of streptomycin were tested for activity after a short period of incubation and found to be exactly the same, whether water or the culture filtrate of an inactive strain was used as a solvent and diluent. In a second experiment, stationary cultures of inactive strains were reinoculated after different periods of incubation with spores of active strains. The rate of glucose consumption and the pH were found to rise rapidly, accompanied by the formation of streptomycin, as shown in table 2. These

TABLE 2

PRODUCTION OF STREPTOMYCIN BY MIXTURES OF INACTIVE AND ACTIVE STRAINS OF *A. griseus*

CULTURE NO.[a]	UNITS/ML. OF FILTRATE, IN DAYS INCUBATION 5	9	11	14	4[b]	6[b]	9[b]	FINAL pH
3	0	0	0	0	..	..	...	5.0
4	0	0	0	0	..	..	...	5.0
3 + 5[c]	83	88	128	86	..	..	...	...
4 + 5[c]	65	85	128	81	..	..	...	...
5	25	48	100	90	..	..	...	8.4
3, followed by 5	..	..	...	..	29	90	128	8.4
4, followed by 5	..	..	...	..	25	90	133	8.4

[a] Cultures 3 and 4 were inactive strains obtained from non-sporulating sectors; culture 5 was an active strain.

[b] Days after reinoculation.

[c] 3 + 5 or 4 + 5 simultaneous inoculation with cultures 3 and 5 or 4 and 5, respectively.

data also reveal that the simultaneous inoculation of a medium with an active and an inactive strain gave virtually the same antibiotic activity as inoculation with an active strain alone. In other words, the inactive strains had apparently no inhibitory effect upon streptomycin production by the active strains or upon the action of streptomycin itself.

The possibility that the initial acidity produced by the inactive strains might inactivate any streptomycin formed was also eliminated, as indicated by the results of another experiment reported in table 3. Addition of the buffering substances, $CaCO_3$ and K_2HPO_4, prevented an acid reaction, but did not result in the formation of streptomycin.

A study was made next of the cycle of growth of the inactive strains in shaken and in stationary cultures. The shaken cultures produced large balls of growth after 2 to 3 days' incubation; however, such cultures gradually developed into a fine, flocculent type of growth that is characteristic of the active strains grown in shaken culture from spore inoculation. The shaken cultures of the inactive strains finally underwent, after 6 to 9 days of incubation, a fairly rapid and complete lysis. It took about twice as long for the submerged growth of active strains to undergo lysis. In the stationary cultures of the inactive strains, no rapid lysis occurred;

TABLE 3

EFFECT OF BUFFER UPON THE PRODUCTION OF STREPTOMYCIN, SHAKEN CULTURES, 4 DAYS OF INCUBATION

STRAIN USED	GLUCOSE LEFT,[a] MG.	STREPTOMYCIN UNITS/ML.	pH
No. 19	0	34	7.8
No. 19 + variant 4	0	34	7.9
Variant 4	175	0	5.2
Variant 4 + $CaCO_3$[b]	0	0	7.4
Variant 4 + K_2HPO_4[b]	44	0	7.1

[a] 1 gm. in control.

[b] 5 gm. per liter.

these cultures consisted of submerged flocculent masses of growth on the bottom of the flask often accompanied by a surface ring around the glass wall. This type of growth persisted even after 30 days' incubation. Upon inoculation of stationary cultures of inactive strains with spores of an active strain, the submerged flocculent growth of the former underwent lysis as the characteristic pellicle of the latter developed.

When the vegetative growth taken from the subsurface portions of colonies of the active strains growing upon an agar plate was inoculated into stationary flasks, the submerged type of growth was obtained. It was similar in every respect to growth of an inactive strain; the pH of the medium was lowered, the glucose consumption was slow, and the filtrate was inactive. Upon the reinoculation of such cultures with spores of an active strain, the pH and rate of glucose consumption rose; the filtrate became active, and the submerged growth gradually lysed as a pellicle developed. An active strain was thus induced to behave like an inactive, non-sporulating strain when the vegetative growth was used as the inoculum. Somewhat analogous results were obtained in shaken cultures when the vegetative growth was used as the inoculum.

To determine whether the production of streptomycin is associated with the sporulating form of the organism, an attempt was made to reisolate sporulating strains, that is, strains producing aerial mycelium, from the non-sporulating variants. These reverted strains were obtained from an occasional old stationary culture of an inactive strain, which developed spontaneously one or more colonies producing sporulating sectors. Transfers made from such sectors yielded cultures which sporulated and produced streptomycin.

It has been shown elsewhere[7] that of various actinomycetes, the streptomycin-producing strain of *A. griseus* is most resistant to the effect of this substance. This method of approach was utilized for the purpose of establishing any genetic differences between the non-sporulating and the sporulating strains of *A. griseus*. For purposes of comparison, several independently isolated cultures of this organism were compared with the

various strains obtained from the streptomycin-producing culture (table 4). The two old cultures of *A. griseus*, namely, Nos. 3326*a* and 3378 not producing any streptomycin, were found to be highly sensitive to the antibiotic effect of this material. On the other hand, the streptomycin-producing culture of *A. griseus*, as typified by the active strains Nos. 4 and 19, were highly resistant to the action of this antibiotic. However, the non-sporulating, inactive variants Nos. 3, 4 and 6 were sensitive to streptomycin. The reverted sporulating form obtained from the non-sporulating variant produced streptomycin and was resistant to its action.

Of particular interest in the above experiment, is variant 6. This variant is intermediate with respect to the active sporulating strains and the inactive non-sporulating strains. In its growth on agar slants it slowly forms a limited amount of aerial mycelium. In liquid media, it produces

TABLE 4

EFFECT OF STREPTOMYCIN UPON THE GROWTH OF DIFFERENT STRAINS OF *A. griseus* AND UPON VARIANTS OBTAINED FROM THE SAME STRAIN

CULTURE OR STRAIN OF *A. griseus*	ORIGIN	PRODUCTION OF STREPTOMYCIN[a]	INHIBITION OF GROWTH[b]
Culture 3326*a*	Original isolation of 1916	0	<3.1
Culture 3378	Later isolate from soil	0	<3.1
Strain No. 4	Sporulating active form	38	>3, 125
Strain No. 19	Sporulating active form	128	>3, 125
Variant 3	Non-sporulating form	0	19.7
Variant 4	Non-sporulating form	0	15.6
Variant 6	Non-sporulating form	4	27.2
Reverted strain from variant 4	Sporulating active form	37	>3, 125

[a] Units of streptomycin in 12-day cultures.

[b] Units of streptomycin required to inhibit growth in 1 ml. of medium.

at first a typical submerged growth characteristic of the non-sporulating strains, and subsequently forms some surface growth which tends to sporulate, although less rapidly and less abundantly than the active sporulating strains. It falls between the two in its capacity to produce a small amount of streptomycin, which results only after the surface growth begins. Finally, it is somewhat more resistant than the true non-sporulating variants 3 and 4 to the antibiotic action of streptomycin. Variant 6 thus combines the capacities of both the sporulating and the non-sporulating strains, being intermediate in its cultural and biochemical characteristics.

A summary of the properties of the active strain and the inactive variant of *A. griseus* is presented in table 5.

Discussion.—The results of experiments on the variation of a certain strain of *Actinomyces griseus* are reported. A certain strain of this organism, having the capacity of producing the antibiotic substance streptomycin, was separated into at least two types; these varied in their mor-

phology, namely, formation of an aerial mycelium, and in their physiology, such as production of antibiotic substance, formation of acid, rate of glucose consumption, autolysis, and production of a gum-like material. Between these two extremes intermediary strains were obtained from which either of the first two types could be readily isolated.

It is to be recalled, in this respect, that the major basis for the separation

TABLE 5

CULTURAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE STREPTOMYCIN-PRODUCING STRAIN OF *A. griseus* AND ITS INACTIVE VARIANT

ACTIVE STRAIN	INACTIVE VARIANT
1. *Antibiotic activity.* Produces streptomycin in both shaken and stationary cultures.	1. No streptomycin formed either in shaken or stationary cultures.
2. *Growth.* Surface growth always heavily sporulated; grayish-green aerial mycelium.	2. No sporulating aerial mycelium; scant development of aerial hyphae with slight tendency to form spores in some old cultures.
3. *Reaction.* Medium always changes to alkaline; pH 7.5–8.5.	3. Reaction of medium at first acid, pH 5.0–6.5; later becoming alkaline.
4. *Glucose.* Glucose completely consumed in 6–8 days in stationary cultures and in 3–4 days in shaken cultures.	4. Glucose utilized more slowly.
5. *Lysis in shaken cultures.* Shaken cultures produce very fine flocculant growth, tending to lyse slowly after about 15 days.	5. Cultures produce at first balls of growth which change into the turbid, flocculant type; rapid and complete lysis in 7–10 days.
6. *Lysis in stationary cultures.* Surface pellicles stable; any submerged, flocculant growth tends to lyse as the surface pellicle develops.	6. Stationary cultures produce no surface growth but flocculant, submerged mycelial growth which lyses slowly, only after a month or longer.
7. *Viscosity.* Culture filtrate not showing any viscosity.	7. Culture filtrate becomes viscous during or after lysis.
8. *Reinoculation.* Inoculation of cultures with lysed inactive culture induces no lysis or reduction in activity.	8. Inoculation of cultures with spores of active strain produces growth and antibiotic activity if some glucose remains.
9. *Variation.* Sporulating strain gives rise to non-sporulating variants.	9. Asporogenous variants may reconvert to active, sporogenous forms.
10. *Sensitivity to streptomycin.* Very resistant to this antibiotic.	10. Very sensitive to this antibiotic.

of two of the four important genera of the *Actinomycetaceae*, *Streptomyces* and *Nocardia*, is the production of an aerial mycelium: the first genus produces such mycelium, whereas the second produces none or only a limited quantity.[9] The fact that a single strain representing a typical *Streptomyces*, namely *A. griseus*, can be made to yield a mutant or variant which has all the characteristics of a *Nocardia* suggests the logical question:

To what extent do the various species of *Nocardia* isolated from natural substrates represent degenerate species or accidental forms of the *Streptomyces* group?

Other significant facts brought out in these studies are the marked physiological properties that characterize different types of actinomycetes. This is contrary to the general assumption in regard to the paucity of certain stable physiological reactions for the characterization of these organisms. A freshly isolated strain of *A. griseus* having the capacity of producing streptomycin formed typical aerial mycelium, characteristic of the species. It changed the reaction of a glucose-containing medium to alkaline, produced characteristic types of surface and submerged growth, underwent only limited lysis, and was markedly resistant to the antibiotic action of streptomycin. On the other hand, the non-sporulating variant produced no aerial mycelium, formed no streptomycin, was sensitive to the antibiotic action of this substance, was characterized by typical growth, which in shaken culture underwent rapid lysis, and produced acid in the glucose-containing medium. Both strains possessed otherwise the various cultural properties which are characteristic of the *A. griseus* species as a whole, such as lack of pigmentation in organic media and also proteolytic and diastatic properties. The non-sporulating strain, when isolated as such, however, would hardly be recognizable as typical *A. griseus*.

Summary.—A strain of *Actinomyces griseus* characterized by the production of the antibiotic substance streptomycin was found capable of variation. Some of the variants produced no aerial mycelium and no streptomycin and were characterized by other properties which distinguished them from the original culture.

Sporulating and streptomycin-producing strains comparable in many respects to the original culture could be isolated under certain conditions of cultivation from the non-sporulating variants.

The original culture of *A. griseus* had all the properties of the newly created genus *Streptomyces;* however, the variant could be classified within the genus *Nocardia.*

The question is, therefore, raised: to what extent may many of the species of *Nocardia* described in the literature represent variants of *Streptomyces* species that have lost the property of producing aerial mycelium?

* Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Microbiology.

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ON EPINEPHRINE AND RETINAL PHOTOMECHANICAL RESPONSES

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When the eyes of many vertebrates (particularly fishes, amphibians and birds) are exposed to light, the retinal pigment migrates, the cones contract and the rods elongate. In darkness the inverse changes occur, viz., contraction of the pigment, elongation of the cones and retraction of the rods. These so-called photomechanical responses (especially in the lower forms) have been regarded as important in adapting the retina to changes in illumination.[1–4] In man and other mammals where these responses apparently fail, adaptation to changing illumination must be sought primarily in the physiology of the retinal photopigments in combination with pupillary responses.

Photomechanical responses have been shown to be influenced by factors other than light and darkness.[2] An important factor, among others, is temperature. Both in fishes and in amphibia, low and high temperatures in the dark will bring about a rather characteristic light response.[2, 5, 6]

Numerous investigators[7–11] have shown that the injection of commercial epinephrine hydrochloride into dark-adapted frogs will produce an expansion of the pigment (light condition) in the dark. This response has been brought into question by Nover,[12] who claimed that, in reality, the migration of the pigment in the dark is not due to the hormone itself but to its acidity. In this respect he supported the acid stimulation theory of Dittler[13] and its amplification by v. Studnitz.[14] The latter maintains that light breaks down cone-photosensitive substance with the production of phosphoric acid, and that the acid initiates the pigment migration and positional changes in the photoreceptors. This theory has received ardent support by Wigger[15] and Nover,[12] and the whole matter of retinal photomechanical shifts is brought into direct dependence upon the H-ion con-

centration (acidity producing pigment expansion and cone contraction; alkalinity producing the inverse changes).

Nover[12] claims that when commercial adrenalin ("Suprarenin Hydrochlor.") is neutralized to pH 6.98 with NaOH and injected into frogs, it has no more effect than the injection of saline solution of the same pH. Consequently the hormone is regarded as ineffectual.

The results of the experiments herewith presented do not support Nover's findings. These experiments were performed upon frogs which had been

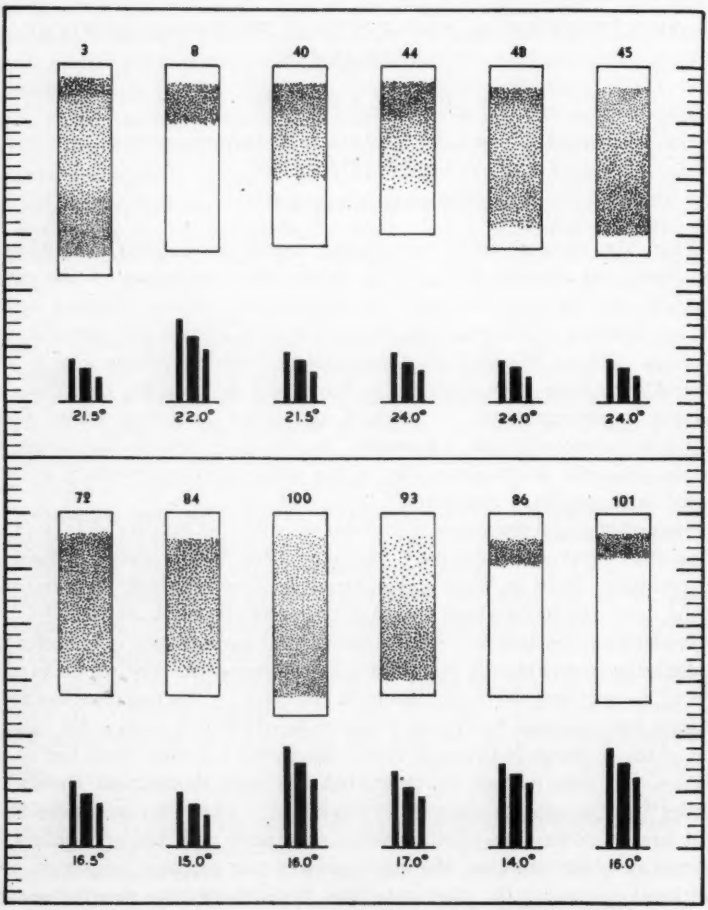

FIGURE 1

(See opposite page for explanation)

kept in darkness for approximately 2 hours—a period sufficient to produce a contracted state of the pigment and elongation of the cones (Fig. 1, case 8). After dark-adaptation the frogs were injected with epinephrine hydrochloride ("Adrenalin"; Parke, Davis and Co.) under conditions stated in table 1. The results upon the pigment and cones are given in figure 1. It becomes clear from a study of these results that epinephrine, whether markedly acid (pH 3.6–4.8) or brought up to neutral or slightly beyond with carbonate (pH 7–7.4), produces a marked migration of the pigment in the dark. Furthermore, injections of the pure base suspended in peanut oil produced the most striking pigment expansion. In most of

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EXPLANATION OF FIGURE 1

Graphic representation of the responses of the frog retinal pigment and cones in various experiments, the protocols of which are given below. The data are partially listed in table 1. The vertical rectangles represent segments of the retina from the base of the epithelial cell (above) to the external limiting membrane (below). The pigment is represented by dots, which show the extent of migration or contraction, as well as density. The lengths of the cones are represented by the vertical solid black columns. The left narrow column for each eye represents the lengths of the longest cones (principal member of double cones); the right narrow column represents the lengths of the shortest cones (single cones). The broad middle column represents the mean length based upon 20 measurements for each retina (10 double and 10 single). Cone measurements are based on the distance in μ from the external limiting membrane to the outer segment (distal margin of the oil globule). Each space on the ordinates equals 4 μ.

Frog 3. Light-adapted 3 hours.

Frog. 8. Dark-adapted 2 hours.

Frog 40. Dark-adapted 2 hours. Injected with 1 cc. of Ringer's solution (pH 6.5). Re-dark-adapted 40 minutes. Eyes excised.

Frog 44. Dark-adapted 2 hours. Injected with 1 cc. of Ringer's solution (pH 6.5). Re-dark-adapted 20 minutes. Eyes excised.

Frog 48. Dark-adapted 2 hours, 30 minutes. Injected with 1 cc. of epinephrine hydrochloride (1:100,000). Re-dark-adapted 30 minutes. Eyes excised.

Frog 45. Dark-adapted 2 hours. Injected with 1 cc. of epinephrine hydrochloride (1:100,000). Re-dark-adapted 20 minutes. Eyes excised.

Frog 72. Dark-adapted 2 hours, 40 minutes. Injected with 1 cc. of epinephrine hydrochloride (1:50,000), neutralized to pH 7.4. Re-dark-adapted 30 minutes. Eyes excised.

Frog 84. Dark-adapted 2 hours, 10 minutes. Injected with 1 cc. of epinephrine hydrochloride (1:50,000), neutralized to pH 7.3. Re-dark-adapted 40 minutes. Eyes excised.

Frog 100. Dark-adapted 2 hours, 15 minutes. Injected with 0.5 cc. of epinephrine in peanut oil (1:500) 1 mg. Re-dark-adapted 2 hours, 30 minutes. Eyes excised.

Frog 93. Dark-adapted 2 hours, 10 minutes. Injected with 0.5 cc. of epinephrine in peanut oil (1:500) 1 mg. Re-dark-adapted 2 hours, 30 minutes. Eyes excised.

Frog 86. Dark-adapted 2 hours, 10 minutes. Injected with 0.5 cc. of peanut oil. Re-dark-adapted 3 hours. Eyes excised.

Frog 101. Dark-adapted 2 hours, 10 minutes. Injected with 0.5 cc. of peanut oil. Re-dark-adapted 2 hours, 30 minutes. Eyes excised.

TABLE 1

SHOWING EFFECTS OF INJECTION OF EPINEPHRINE HYDROCHLORIDE INTO THE VENTRAL LYMPH SAC OF DARK-ADAPTED FROGS, AND RE-DARK-ADAPTING FOR PERIODS INDICATED

SUBSTANCE INJECTED	pH OF SOLUTION	CASE	RE-DARK-ADAPTED	TEMP. IN DEG. C	EXTENT OF PIGMENT MIGRATION	LENGTH OF CONES* LONGEST	SHORTEST	MEAN
1 cc. of 1:100,000 sol. epineph. hydrochlor.	...	45	20 min.	24.0	++++	15.6	9.6	12.4
	...	48	30 min.	24.0	+++	15.6	9.6	12.7
	...	50	20 min.	24.0	+++	20.4	14.4	17.4
1 cc. of 1:50,000 sol. epineph. hydrochlor.	4.8	76	35 min.	16.5	++++	22.8	15.6	18.8
	3.6	85	35 min.	15.0	+++	..	..	..
	7.4	72	30 min.	16.5	+++	22.8	15.6	19.4
	7.3	83	40 min.	15.0	+++	18.0	10.8	14.8
	7.3	84	40 min.	15.0	+++	19.2	10.8	14.9
1 cc. of 1:25,000 sol. epineph. hydrochlor.	7.0	104	40 min.	13.0	+++	..	..	..
	7.0	105	40 min.	13.0	+++	25.2	16.8	21.4
Epinephrine suspension in peanut oil								
0.5 cc. of 1:500 (1 mg.)	Neutral	93	2 hrs. 30 min.	17.0	++++	26.4	18.0	21.7
		94	2 hrs. 30 min.	17.0	++++	30.0	19.8	23.9
		100	2 hrs. 30 min.	16.0	++++	36.0	24.0	30.2
0.25 cc. of 1:500 (0.5 mg.)		102	2 hrs. 30 min.	16.0	++++	34.8	21.6	29.1
		103	2 hrs. 30 min.	16.0	++++	39.6	25.2	34.3
0.5 cc. of peanut oil	Neutral	80	40 min.	14.5	+	22.8	14.4	18.5
		86	3 hrs.	14.0	−	30.0	22.8	26.4
		101	2 hrs. 30 min.	16.0	−	36.0	25.2	30.7

++++ = Extensive migration with distal accumulation of pigment (e.g., case 45, Fig. 1).
+++ = Extensive migration with no distal accumulation (e.g., case 48, Fig. 1).
++ = Pigment migrated between one-third and two-thirds down (e.g., case 40, Fig. 1).
+ = Very slight migration of pigment.
− = No migration. Typical dark condition (e.g., case 86, Fig. 1).

* Longest cones are principal members of double cones. Shortest cones are single cones. Mean length is based on average of 20 measurements for each case.

these latter cases the pigment became heavily massed near the external limiting membrane (e.g., case 93, Fig. 1). The cones, however, in many of these retinas were as long as those in the retinas of control frogs, i.e., those injected with peanut oil alone where, after $2^1/_2$ to 3 hours in darkness, the pigment was found to be maximally contracted and the cones elongated (Fig. 1, cf. cases 93 and 100 with cases 86 and 101). These results indicate clearly that pigment migration and cone contraction do not always go together.

It has been shown earlier[16] that any excitation of the frog in the dark will produce a partial light response of the retina in darkness. It has been suggested that this response may be due to the increased secretion of the adrenal medulla resulting from excitation. In the present experiments this tentative theory would seem to be supported inasmuch as injections of epinephrine produce marked pigmentary responses in the dark. That the adrenal gland exerts a definite influence on retinal pigmentary responses has been shown by Hasama.[17] He found that after cauterization of the frog adrenal gland, the pigment failed to expand upon exposure to light. Furthermore, faradic stimulation of the adrenals in dark-adapted frogs was shown to produce the light condition of the pigment.

Summary.—When commercial epinephrine hydrochloride ("Adrenalin"; Parke, Davis and Co.) is injected into dark-adapted frogs, the retinal pigment undergoes marked expansion in the dark. This ensues whether the solution is acid (pH 3.6–4.8) or is neutralized with carbonate to pH 7. Injections of the pure epinephrine base suspended in peanut oil produce maximal migration in $2^1/_2$–3 hours. When peanut oil alone is injected, the pigment, after equivalent exposures to darkness, is in a highly contracted state. The results indicate that the pigment expansion is due to the action of the hormone itself, and not the result of acid stimulation.[18]

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[16] Detwiler, S. R., *Jour. Comp. Neur.*, **81**, 137–145 (1944).
[17] Hasama, B., *Pflüger's Arch.*, **238**, 758–766 (1937).
[18] A detailed account of the effect of injections of acids and alkalis upon retinal pigmentary responses in the frog will be published elsewhere.

THE VARIATION OF INFECTIVITY

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There are two main approaches to the study of the epidemic curve of new cases. First is what may be called the dynamical approach in which *a priori* assumptions are made as to the laws regulating the relations between the changing case rates and the number of susceptibles; Ronald Ross, Soper, McKendrick and others have followed this line of attack. Second is what may be called the statistical approach in which some form is assumed for the curve of new cases, generally based *a posteriori* on the observation that such a curve does describe fairly well the course of new cases; Farr, Brownlee and others have pursued this method.

We desire to examine the two methods together to see what conclusions result. The three curves which have been most used are: (1) the probability curve

$$C = \frac{T}{\sqrt{2\pi}\,\sigma}\, e^{-\frac{t^2}{2\sigma^2}}, \tag{1}$$

where T is total cases during the whole epidemic, t is the time in any unit, σ the standard deviation of the distribution of the case rates in time, and C is the case rate of new cases at the time t; (2) Pearson's Type IV, much used by Brownlee in its general form, which we shall use only in its symmetrical form[1] (Type VII), namely,

$$C = \frac{T\Gamma(n)}{a\sqrt{\pi}\,\Gamma(n - {}^1/_2)}\left(1 + \frac{t^2}{a^2}\right)^{-n}, \tag{2}$$

where $a = (2n - 3)^{1/2}\sigma$ and Γ is the symbol of the gamma-function; (3) the derivative of the growth curve, namely

$$C = {}^1/_2 T\beta\,\mathrm{sech}^2\,\beta t, \tag{3}$$

where $\beta = \pi/(2\sqrt{3}\,\sigma) = 0.9069/\sigma$. Each of these symmetrical curves has been referred to its mean.

The dynamical law which we shall use is the law of mass action as we have recently generalized it,[2] viz.,

$$C(t) = \left(\frac{S}{m}\right)^p C(t - \tau), \tag{4}$$

for a disease of definite incubation time τ. The number m, which is the number of susceptibles just sufficient so that the case rate is the same at t as at $t - \tau$, can be taken as *one* measure of the reciprocal of the infectivity. The infectivity thus defined is a strictly epidemiological concept and has no intimate connection with bacterial virulence. Given any curve of new cases, we have

$$m = S[C(t - \tau)/C(t)]^{1/p}. \tag{5}$$

The value of S is not known but if S_0 be the number of susceptibles at the peak of the epidemic curve

$$S = S_0 - \int_0^t C\,dt \tag{6}$$

and the values of S at the beginning and the end of the epidemic are

$$S_B = S_0 + T/2, \qquad S_E = S_0 - T/2 \tag{7}$$

and the value of m at the peak is very nearly

$$m_0 = S_0 - {}^1/_2 \text{ peak cases} \tag{8}$$

where peak cases are defined as the number of cases that would occur during the period τ if the peak case rate were maintained throughout that interval of time.

Brownlee defines infectivity[3] directly from the epidemic curve without reference to the number of susceptibles as

$$\frac{C + \Delta C}{C} = \frac{C(t + \tau)}{C(t)} = f(t). \tag{9}$$

From this it appears that, if $p = 1$ in the dynamical law, his definition makes

$$f(t) = \frac{S(t - \tau)}{m(t - \tau)}, \qquad \text{or } f(t + \tau) = \left(\frac{S}{m}\right)^p \tag{10}$$

in the general case. Actually, like many workers, he assumes that τ is short enough so that differentials may replace increments and

$$f(t) = e^{\tau(d \log C/dt)}. \tag{11}$$

If the three curves (1)–(3) be taken as the epidemic curve, the infectivity as defined by Brownlee in (11) gives, respectively,

$$f(t) = e^{-\tau t/\sigma^2} \quad \text{or } e^{-\frac{2n\tau t}{a^2 + t^2}} \quad \text{or } e^{-2\beta\tau \tanh \beta t}.$$

For t large and negative the results are ∞, 1, $e^{2\beta\tau}$; whereas for t large and positive they are 0, 1, $e^{-2\beta\tau}$; and at the peak of the epidemic ($t = 0$) they are[4] 1, 1, 1.

If m be taken as a measure of the reciprocal of the infectivity and equation (5) be used, the results are somewhat more complicated but not too dissimilar. One may begin with (3), whence

$$m = \frac{\operatorname{sech}^{2/p} \beta\tau(S_0 - {}^1/_2 T \tanh \beta t)}{(1 - \tanh \beta\tau \tanh \beta t)^{2/p}}.$$

If $S_0 : T/2 = \coth \beta\tau$ the numerator and denominator become proportional and the value of m increases throughout the epidemic, i.e., the infectivity $1/m$ falls off, provided $p < 2$; but the opposite holds provided $p > 2$; and for $p = 2$ the value of m is constant. If, however, $S_0 : T/2 \neq \coth \beta\tau$ the relations become more complicated; in the special case $p = 2$ the value of m will increase throughout the epidemic if $S_0 : T/2 > \coth \beta\tau$ but will decrease if $S_0 : T/2 < \coth \beta\tau$.

If attention be turned to the normal curve (1),

$$m = \left(S_0 - \frac{T}{\sqrt{2\pi}\,\sigma} \int_0^t e^{-\frac{t_3}{2\sigma^2}} dt \right) e^{\tau(2t - \tau)/2p\sigma^2}.$$

When $t = -\infty$, $m = 0$; when $t = 0$, $m = S_0 e^{-\tau^2/2p\sigma^2}$; when $t = +\infty$, $m = \infty$ provided S_E does not become zero and it generally does not. Although m is greater when $t = 0$ than when $t = -\infty$ and less when $t = 0$ than when $t = \infty$ no matter what the value of t, the rate of change of m when $t = 0$ will be negative if

$$p > \sqrt{2\pi}\,\frac{\tau}{\sigma}\,\frac{S_0}{T} = 2.507\,\frac{\tau}{\sigma}\,\frac{S_0}{T}.$$

As S_0 need not be larger than $T/2$ and as τ/σ is generally less than $^1/_2$ when the epidemic runs for a considerable number of incubation periods, it is clear that p need not be very large to satisfy the relationship; and when the relationship is satisfied m starts at 0 when $t = -\infty$, increases to a maximum prior to the peak of the epidemic, decreases to a minimum after the peak, and then increases indefinitely.

The sort of results that arise on combining the generalized law of mass action with specific assumptions as to the type of epidemic curve which may be assumed "as found by trial apart from theory," including that particular type which has been shown to arise by theory as a first approximation (when there are no recruits), may be exhibited better by a specific illustrative example than by detailed development of formulas. Let us take the 1925–1926 epidemic from Hedrich's Baltimore series. Table 1 gives the estimated cases by months, the cases that would be calculated from curves of types (1), (2), (3) fitted to the data, and the values of the infectivity taken as $70604/m$ computed from these curves (not from the data) for the hypotheses $p = 1, 2, 4$. The value assumed for S_0 at the mode was 72982 and the equilibrium value was figured as 70604. It will

TABLE 1

THE VARIATION OF THE INFECTIVITY UNDER A VARIETY OF HYPOTHESES

TIME	CASES	(1)[3]	(2)[4]	(3)[5]	VALUE[6] OF $\frac{70604}{m}$ FROM (1) $p=1$	$p=2$	$p=4$	B[7]	VALUE OF $\frac{70604}{m}$ FROM (2) $p=1$	$p=2$	$p=4$	B	VALUE OF $\frac{70604}{m}$ FROM (3) $p=1$	$p=2$	$p=4$	B
$-\infty$	...[2]	0	0	0	∞	∞	∞	∞	0.80	0.80	0.80	1.00	1.49	1.09	0.93	1.87
Aug.	50	3	24	27	3.24	1.61	1.13	4.48	0.90	0.85	0.82	1.65	1.49	1.09	0.93	1.87
Sept.	72	44	104	103	2.60	1.44	1.07	3.53	0.91	0.85	0.82	1.73	1.48	1.09	0.93	1.87
Oct.	222	308	326	354	2.35	1.37	1.04	2.80	0.92	0.86	0.83	1.81	1.48	1.09	0.93	1.86
Nov.	1379	1412	1127	1177	1.86	1.22	0.99	2.20	0.93	0.86	0.83	1.86	1.47	1.08	0.93	1.83
Dec.	2602	4056	3490	3505	1.49	1.10	0.94	1.73	0.94	0.87	0.84	1.79	1.44	1.08	0.94	1.75
Jan.[1]	8370	7390	7748	7682	1.23	1.02	0.93	1.36	0.95	0.89	0.87	1.53	1.35	1.07	0.95	1.52
Feb.[1]	10931	8492	9540	9496	1.07	1.00	0.96	1.08	0.98	0.95	0.94	1.12	1.14	1.03	0.98	1.12
Mar.[1]	5060	6188	6022	5981	0.95	1.00	1.02	0.85	1.02	1.04	1.05	0.78	0.90	0.98	1.02	0.77
Apr.	1881	2843	2304	2344	0.82	0.98	1.06	0.67	1.04	1.10	1.14	0.60	0.75	0.93	1.04	0.61
May	708	825	705	742	0.68	0.91	1.05	0.53	1.05	1.13	1.17	0.55	0.69	0.91	1.05	0.56
June	209	152	206	219	0.54	0.82	1.00	0.41	1.05	1.14	1.18	0.54	0.67	0.91	1.06	0.54
July	168	19	66	64	0.43	0.73	0.95	0.33	1.06	1.15	1.19	0.56	0.66	0.91	1.06	0.54
Aug.	81	0	20	21	0.35	0.66	0.90	0.26	1.08	1.16	1.19	0.59	0.66	0.91	1.06	0.54
$+\infty$	...[2]	0	0	0	0	0	0	0	1.23	1.23	1.23	1.00	0.66	0.91	1.06	0.53

[1] The months of Jan., Feb., Mar. were adjusted to 30 days; the unit of time was taken as a half-month.

[2] The actual epidemic cannot well be followed past the epidemic year in either direction.

[3] The fitted curve (1) was taken to be $C = 4370\, e^{-t^2/17.78}$ with mode about Feb. 9.

[4] The fitted curve (2) was taken to be $C = 4990\,(1 + t^2/41.95)^{-4}$ with mode at the same time.

[5] The fitted curve (3) was taken to be $C = 4968\ \mathrm{sech}^2\,(0.3131t)$ with mode at the same time.

[6] The values are those for the first of the month; the value of 70604 is the "equilibrium" number m_0.

[7] The infectivities under B are those computed from (11) rather than from (9).

be observed that for each type of curve the fit to the data is rather poor and that, therefore, values of the infectivity calculated directly from the original data would undoubtedly behave differently from those based on any of the fitted curves. However, this table is offered for the purpose of showing how the infectivity would theoretically vary if these types of curve were supposed to hold precisely for an epidemic.

The following comments may be made on Table 1. Brownlee's infectivity decreases for (1) and (3) throughout the course of the epidemic curve, but for (2) it shows at the very ends (August) the reversal which has been noted in the theoretical discussion.[4] For the normal curve (1) the infectivity defined as proportional to the reciprocal of m and standardized to be unity when S has its equilibrium value m_0 and $C(t) = C(t - \tau)$, is highly variable if $p = 1$ or $p = 2$ (but only moderately variable if $p = 4$) within the natural limits of the epidemic; it decreases throughout the epidemic if $p = 1$ or $p = 2$ but shows the fluctuation near the mode if $p = 4$ as described in the text. For the curve (2) the infectivity increases throughout the epidemic being not very variable for $p = 1$ but increasingly so for $p = 2$ and $p = 4$. For the curve (3) there is a decreasing infectivity with considerable variation if $p = 1$ and with little variation if $p = 2$, whereas if $p = 4$ there is an increasing infectivity with slight variation; clearly for some value of p intermediate between 2 and 4 there would be practical (though not quite absolute) constancy in the infectivity. It is certain that whether the infectivity be defined as by Brownlee or whether it be taken from the generalized law of mass action, its quantitative (and even its qualitative) behavior is very different according to the type of epidemic curve selected.

[1] *Encyclopedia Britannica*, 14th ed., Vol. 8, p. 650. In this article on Epidemiology, Brownlee and Greenwood state: "The equation of the curve which describes the majority of epidemics, as found by trial apart from theory, is $y = a(1 + t^2/b^2)^{-n}$—(Hypothetically) the organism may be assumed to possess at the beginning of the disease a high degree of infectivity which decreases as the epidemic goes on." We may observe that Brownlee fits Type IV from four moments of the epidemic curve. There is no difficulty about this if the epidemic is that of a disease which starts and stops abruptly but there are real difficulties in finding reliable results for third and fourth moments in many cases where the beginning and end of the epidemic are somewhat indefinite. Further, for Type VII, $\beta_2 > 3$, the relation between n and β_2 being

$$n = (5\beta_2 - 9)/(2\beta_2 - 6) \quad \text{or} \quad \beta_2 = (6n - 9)/(2n - 5)$$

so that for $n = \infty$ we have $\beta_2 = 3$ (the normal curve) and n must be greater than $5/2$ for which β_2 would become infinite. For the derivative of the growth curve $\beta_2 = 4.2$ and Type VII fitted thereto by moments would need $n = 5$.

[2] These PROCEEDINGS, **31**, 109–116 (1945).

[3] John Brownlee, "On the Curve of the Epidemic," *British Med. Jour.*, **1**, 799–800 (1915).

[4] If (1) or (3) be used it is clear that $f(t)$ always decreases; but for (2)

$$f'(t) = 2n\tau e^{-\frac{2n\tau t}{a^2 + t^2}}\left[\frac{t^2 - a^2}{(a^2 + t^2)^2}\right]$$

so that $f(t)$ increases from 1 when $t = -\infty$ to a maximum of $e^{n\tau/a}$ when $t = -a$, decreases to a minimum of $e^{-n\tau/a}$ when $t = +a$, and then increases to 1 when $t = +\infty$. The value of C as a fraction of its modal value C_0 at the time $t = a$ is 2^{-n} so that the infectivity according to Brownlee's definition is increasing up to the time when the case rate is $C_0/2^n$, then decreases to the time when again the case rate is $C_0/2^n$, and finally increases thereafter. If n is large the tails of the curve beyond the place where $C = C_0/2^n$ may be disregarded so that practically the statement of Brownlee and Greenwood that the infectivity decreases from beginning to end of the epidemic is true, but if n is large the statement cannot be quite true of Brownlee's definition and this epidemic curve.

GROUPS HAVING A SMALL NUMBER OF SETS OF CONJUGATE SUBGROUPS

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The identity and the group itself are sets of conjugate subgroups of every group G. If G is the cyclic group of order p^m, p being an arbitrary prime number, then G contains exactly $m + 1$ sets of conjugate subgroups and when p is odd this cyclic group can be extended by p^m subgroups of order q, where q is an arbitrary prime divisor of $p - 1$, so as to obtain a group of order $p^m q$ which contains exactly $m + 3$ sets of conjugate subgroups. In particular, when $m = 1$ the group of order p contains exactly two sets of conjugate subgroups and this is the only group which contains exactly two sets of conjugate subgroups. The two groups of order pq contain separately exactly four sets of conjugate subgroups. One of these is cyclic and the other is non-cyclic and contains p subgroups of order q.

The cyclic group of order p^2 clearly contains exactly three sets of conjugate subgroups and this is the only group which contains exactly three sets of conjugate subgroups. This results from the facts that if a group contains exactly three sets of conjugate subgroups its order cannot be divisible by two distinct prime numbers and the central quotient group of every non-abelian prime power group is non-cyclic. If a group contains exactly four sets of conjugate subgroups its order cannot be divisible by more than two distinct prime numbers and when it is devisible by two distinct prime numbers the order is the product of these numbers.

From what precedes it results that if a group does not contain more than four sets of conjugate subgroups it is the identity if it contains one and

only one such set, it is a group of prime order if it contains two and only two such sets, it is the cyclic group whose order is the square of a prime number if it contains three and only three such sets, and it is either one of the two possible groups whose orders are the product of two distinct prime numbers or the cyclic group whose order is the cube of a prime number when it contains four and only four such sets. In all of these cases, except the first, the number of the possible groups is infinite and all of these groups are abelian except the groups of order pq which certain p subgroups of order q.

Before considering all the groups which contain five and only five sets of conjugate subgroups it may be desirable to consider the prime power groups with respect to the number of sets of conjugate subgroups. The non-cyclic group of order p^2 clearly contains $p + 3$ sets of conjugate subgroups and this is the smallest number of sets of conjugate subgroups in any non-cyclic prime power group. This is obvious when the group is abelian and when it is non-abelian it results from the facts that the central quotient group of a non-abelian group is always non-cyclic and that the sets of conjugate subgroups in a group is always larger than the number of sets of conjugate subgroups in any of its quotient group with respect to a non-identity invariant subgroup. By forming successive central quotient groups we arrive at an abelian group.

From what precedes it results that the only prime power non-cyclic group which contains as few as five sets of conjugate subgroups is the four group. The prime power cyclic group of order p^4 also contains exactly five sets of conjugate subgroups according to the theorem noted above. This is also true of the infinite system of groups of order p^2q which are obtained by extending the cyclic group of order p^2, p being any odd prime number, by p^2 cyclic groups of order q, where q is an arbitrary prime divisor of $p - 1$. The smallest groups in the latter category are the dihedral group of order 18 and the dihedral group of order 50. The smallest group of this category which is not dihedral is of order 147 and is obtained by extending the cyclic group of order 49 by 49 subgroups of order 3. There is no other group whose order is divisible by as few as two distinct prime numbers in which there are exactly five sets of conjugate subgroups with the exception of the tetrahedral group.

This results almost directly from the fact that all such groups are known to be solvable and hence one arrives at the identity by forming successive commutator subgroups. It is also true that when the order of G is divisible by three distinct prime numbers then G cannot contain exactly five sets of conjugate subgroups. In fact, the order of G could then not be divisible by the square of one of any of these prime numbers and hence G would again be solvable. In this case it would contain an invariant subgroup whose order would be equal to the product of the two largest of these three

prime numbers. This invariant subgroup could not be non-cyclic and hence all of its operators besides the identity would be non-commutative with one of the remaining operators of G. It would therefore result that G would contain more than five sets of conjugate subgroups, which is contrary to the hypothesis. We have therefore considered all the possible groups which separately involve as few as five sets of conjugate subgroups.

If G has six sets of conjugate subgroups and its order is a power of a prime number this number could not exceed 3 when G is non-cyclic because $p + 3$ would then exceed 6. If it is cyclic it is of order p^5, where p is an arbitrary prime number in accord with the general theorem noted above. Since every non-cyclic group of order p^m contains at least $p + 1$ subgroups of index p and these are invariant under the group it results that every non-cyclic group of order p^m contains at least $p + 1$ sets of conjugate subgroups under the group which are separately of index p. Moreover, every group of order p^m contains at least one invariant subgroup of order p. Hence it results that when a group of order 2^m contains exactly six sets of conjugate subgroups it can contain only one subgroup of order 2 and is, therefore, either cyclic or dicyclic. That is, the quaternion group is the only group of order 2^m which contains exactly six sets of conjugate subgroups and is non-cyclic.

If a non-cyclic group of order 3^m contains exactly six sets of conjugate subgroups then $m = 2$ and G is the non-cyclic group of order 9 in accord with the general theorems noted above. That is, *if a prime power group contains exactly six sets of conjugate subgroups it may be the group of order p^5, p being an arbitrary prime number, the quaternion group, or the non-cyclic group of order 9, but it can be no other group.* It remains to consider the possible cases when G contains exactly six sets of conjugate subgroups but its order is not a power of a prime number.

The number of the distinct prime numbers which divide the order of G could clearly not exceed four since the number of the sets of conjugate subgroups of G is supposed to be six. If this former number would be four the order of G would not be divisible by the square of a prime number and hence the main properties of G would be known. In particular, G would have to contain at least two operators of prime orders which would be commutative. As this is impossible the order of G could not be divisible by as many as four distinct prime numbers when it contains exactly six sets of conjugate subgroups.

Suppose that the order of G is divisible by three distinct prime numbers but that the square of one of these prime numbers also divides the order of G. Although the simple group of order 60 satisfies this condition it is easy to prove that if we add the condition that the operators of G are contained in six sets of conjugates then G must be solvable and hence it must contain an invariant subgroup of prime index. The order of this

invariant subgroup could not be divisible by three distinct prime numbers since it would then contain a characteristic subgroup whose order would be the product of the two largest of these prime numbers and hence G would involve subgroups of more than six different orders, which is contrary to the hypothesis. The said invariant subgroup of G could also not be divisible by the square of a prime number for similar reasons.

If the order of G is divisible by three distinct prime numbers but not by the square of one of these numbers it contains an invariant subgroup whose order is the product of the two largest of these three prime numbers. If this invariant subgroup is cyclic it can clearly be extended by an operator which transforms it into itself but is not commutative with any operator of this invariant subgroup besides the identity. In particular, if the order of such a group is even there is always an operator of order 2 in the group which has this property. The group thus obtained clearly contains exactly six sets of conjugate subgroups and the dihedral group of order 30 is the group of smallest order which satisfies these conditions. When the order of G is pq^3 and G involves an invariant subgroup of order q^3 and of type 1^3 which involves no invariant operator besides the identity then G clearly involves exactly six sets of conjugate subgroups. The group of order 56 which contains eight subgroups of order 7 is a well-known illustration of the groups of this category.

The present article is closely related to the one published in these PROCEEDINGS, **30,** 359–362 (1944) under the heading "Groups containing a small number of sets of conjugate operators." It may be noted that in some cases the given number of conjugate subgroups imposes a greater restriction on the possible groups than the given number of conjugate operators since the number of the possible subgroups may be larger than the number of the possible operators in a given group.

THE VELOCITY OF PROPAGATION OF BRITTLE CRACKS IN STEEL

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Recently there have been instances in which steel plate used in a variety of structures and subjected to certain severe conditions has ruptured in a brittle fashion. Characteristics of these brittle failures are (1) a crack is propagated with extreme rapidity through the steel plate, (2) the surface

of rupture created by this crack is orthogonal to the plane of the plate, (3) this rupture surface shows a characteristic "herringbone" pattern and (4) there exist small permanent strains, often of less than 2 per cent, in the neighborhood of the fractured surface.

Many engineering structures have failed in this catastrophic manner. One example which attracted a great deal of attention was the explosion at Schenectady, N. Y., of a spherical storage tank containing hydrogen.[1]

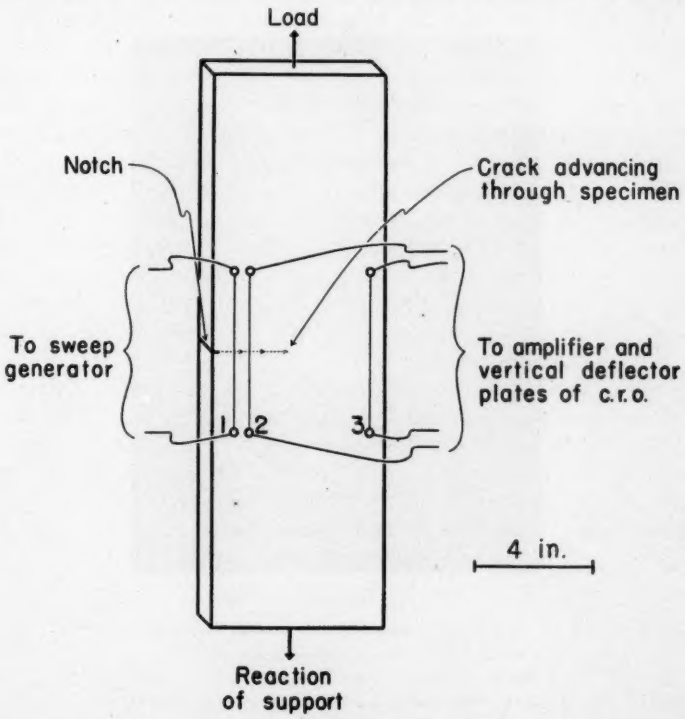

FIGURE 1

Schematic diagram showing the positions of the break-wires *1*, *2* and *3* on the tensile specimen.

Still another example is the breaking up of certain types of welded merchant vessels in a seaway. It is thought that a study of the speed of propagation of brittle cracks in steel will help in understanding the mechanism of these phenomena.

In this note we announce the results of some measurements which have been made of the velocity of propagation at room temperature of brittle

cracks in notched tensile specimens of medium steel. Several fine wires were cemented a few inches apart on the surface of a specimen, and placed normally to the expected path of the crack (Fig. 1). When the specimen is pulled to failure a crack is propagated from the notch across the specimen and breaks the wires in turn. The first wire which breaks triggers a sweep generator which moves a beam of electrons horizontally across the screen of a cathode ray oscillograph. As each succeeding wire breaks, the electron beam jumps vertically resulting in a record like that shown in figure 2.

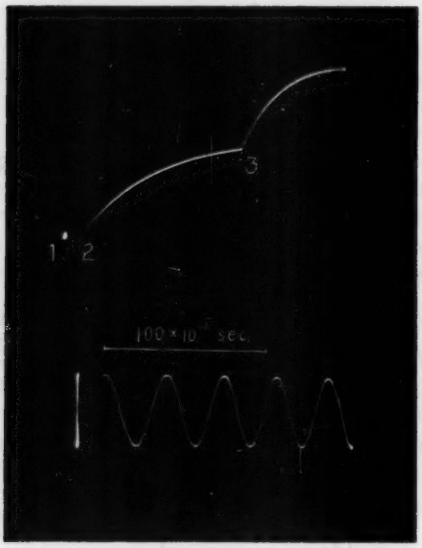

FIGURE 2

A typical record obtained from the breaking of a tensile specimen like that shown in figure 1. The numbers *1*, *2* and *3* designate the positions of the electron beam at the instant of breaking of wires *1*, *2* and *3*, respectively.

The time interval between jumps is measured from the record. This datum combined with the measured distance between the corresponding break-wires enables one to calculate the velocity of the crack. In this way there has been obtained an average velocity of 40×10^3 inches per second with a mean absolute deviation of 6 per cent.

It is expected that a paper giving more details of this work will be published in the near future.

[1] Brown, A. L., and Smith, J. B., *Mech. Eng.*, **66**, 392–397 (1944).